

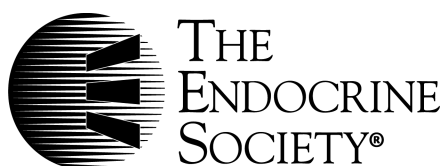
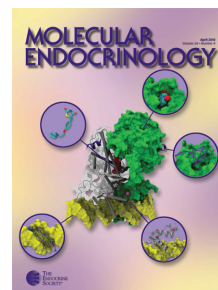
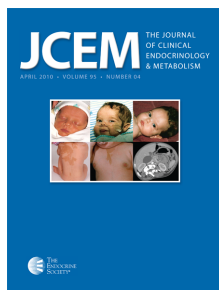
ENDOCRINE REVIEWS

Genetics of Obesity in Humans

I. Sadaf Farooqi and Stephen O'Rahilly

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Genetics of Obesity in Humans

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Considerable attention has focused on deciphering the hypothalamic pathways that mediate the behavioral and metabolic effects of leptin. We and others have identified several single gene defects that disrupt the molecules in the leptin-melanocortin pathway causing severe obesity in humans. In this review, we consider these human monogenic obesity syndromes

and discuss how far the characterization of these patients has informed our understanding of the physiological role of leptin and the melanocortins in the regulation of human body weight and neuroendocrine function. (Endocrine Reviews 27: 710–718, 2006)

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I. Introduction

CHANGES IN DIET and physical activity have driven the rise in the prevalence of adult and childhood obesity over a relatively short time interval (1, 2). However, it is important to recognize the significant contribution of hereditary influences on weight, especially at a time when we are beginning to develop an understanding of the molecules involved in the control of energy homeostasis and how genetic variation within them can influence human obesity (3, 4). The genetic contribution to body weight has been established through family studies, investigations of parent-offspring relationships, and the study of twins and adopted children (5, 6). These studies consistently report heritability estimates of 40–70% (3). Thus, weight is a highly heritable trait, with a heritability that is only slightly less than that for

height (7, 8). As is the case for height, where nutritional changes in the last 50 yr have contributed to substantial increases in mean final height in many populations, environmentally driven changes in body weight in the population occur against a background of susceptibility to weight gain that is determined by genetic factors. Thus, genetic approaches can be applied to understand both the molecular and physiological mechanisms involved in human obesity.

Progress to date has mostly been in more severe familial forms of obesity presenting in childhood. Although these only represent a small fraction of those with obesity (albeit a group with disproportionate physical and psychosocial morbidity and health costs), the implications of these discoveries have been profound (9). We have explored the genetic basis of severe childhood obesity where we considered that major and more highly penetrant genetic effects were likely to be found. In 1997, we established the Genetics of Obesity Study (GOOS) to recruit patients with severe obesity [body mass index (BMI) SD score (SDS) > 3] of early onset (<10 yr). We were particularly interested in children with a strong family history of obesity and those from consanguineous families. Our intention was to use a candidate gene approach to look for mutations in genes thought to play a role in the regulation of body weight based on evidence primarily from rodent models at the time. With the help of colleagues throughout the world, we have to date recruited over 2200 patients to the GOOS cohort. In the past 9 yr, several human disorders of energy balance that arise from genetic defects have been described by ourselves and others (9). All of these are in molecules identical or similar to those known to cause obesity in genetic and experimental syndromes of obesity in rodents and all have been identified using a candidate gene approach. These mutations all result in severe obesity in childhood without developmental pleiotropic features.

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Abbreviations: BDNF, Brain-derived neurotrophic factor; BMI, body mass index; GOOS, Genetics of Obesity Study; MC4R, melanocortin 4 receptor; PC, prohormone convertase; SDS, SD score; TrkB, tropomyosin-related kinase B.

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II. Mutations in Genes Encoding Leptin and the Leptin Receptor

In 1997, we reported two severely obese cousins from a highly consanguineous family of Pakistani origin (10). Both children had undetectable levels of serum leptin and were

found to be homozygous for a frameshift mutation in the *LEP* gene ($\Delta G133$), which resulted in a truncated protein that was not secreted. We have since identified five further affected individuals from four other families (Refs. 11 and 12 and our unpublished observations) who are also homozygous for the same mutation in the leptin gene. All the families are of Pakistani origin but are not known to be related over five generations. A large Turkish family in which three adults carry a homozygous missense mutation (C→T substitution at codon 105 resulting in Arg→Trp) in the *LEP* gene have also been described (13).

To date, only one mutation in the leptin receptor gene has been published (14). The mutation was found in homozygous form in three severely obese adult siblings from a consanguineous family of Algerian origin. This mutation results in abnormal splicing of leptin receptor transcripts and generates a mutant leptin receptor that lacks both transmembrane and intracellular domains. The mutant receptor circulates at high concentrations bound to leptin, resulting in very elevated serum leptin concentrations (15).

III. Clinical Phenotypes Associated with Leptin and Leptin Receptor Deficiency

The clinical phenotypes associated with congenital leptin and leptin receptor deficiencies are similar. Leptin- and leptin receptor-deficient subjects are of normal birth weight but exhibit rapid weight gain in the first few months of life, resulting in severe obesity (11). Body composition measurements show that leptin deficiency is characterized by the preferential deposition of fat mass giving a distinct clinical appearance with excessive amounts of sc fat over the trunk and limbs (Fig. 1) (11). All patients were hyperinsulinemic, consistent with the severity of obesity, and some adults have developed type 2 diabetes in the third to fourth decade (11).

All subjects in these families are characterized by intense hyperphagia with food-seeking behavior and aggressive be-

havior when food is denied (11). Energy intake at an *ad libitum* meal is markedly elevated in leptin- and leptin receptor-deficient subjects.

In leptin-deficient humans, we found no detectable changes in resting metabolic rate using indirect calorimetry or total energy expenditure using chamber calorimetry (16). Free-living energy expenditure measured using the doubly labeled water method was not significantly different after adjustment for body composition. However, Ozata *et al.* (17) reported abnormalities of sympathetic nerve function in leptin-deficient adults consistent with defects in the efferent sympathetic limb of thermogenesis.

Leptin and leptin receptor deficiency are associated with hypothalamic hypothyroidism and hypogonadotropic hypogonadism. Evidence from rodents suggests that leptin is necessary for the normal biosynthesis and secretion of TRH (18). Complete leptin deficiency is associated with a moderate degree of hypothalamic hypothyroidism characterized by low free T_4 and high serum TSH that is bioinactive. In leptin-deficient children, plasma free T_4 concentrations are within the normal range, but four children had significantly elevated TSH levels (11), and the pulsatility of TSH secretion, studied in a single adult with congenital leptin deficiency, was characterized by a markedly disorganized secretory pattern (19). Two subjects homozygous for a mutation in the leptin receptor were diagnosed with hypothyroidism in childhood, and thyroid hormone replacement therapy commenced (14).

Normal pubertal development does not occur in adults with leptin or leptin receptor deficiency, with biochemical evidence of hypogonadotropic hypogonadism (13). However, there is some evidence for the delayed but spontaneous onset of menses in one leptin-deficient and three leptin receptor-deficient adults (17).

Leptin-deficient children have normal linear growth in childhood and normal IGF-I levels. However, because of the absence of a pubertal growth spurt, the final height of adult subjects is reduced. In the one previously reported leptin receptor-deficient family, short stature and abnormal serum levels of GH and IGF binding protein 3 were noted in childhood (14). However, assessment of the GH/IGF axis is difficult in obese children and adults because obesity itself is associated with abnormalities in basal and dynamic tests of the GH/IGF axis.

We demonstrated that children with leptin deficiency had profound abnormalities of T cell number and function (11), consistent with high rates of childhood infection and a high reported rate of childhood mortality from infection in obese Turkish subjects (17).

IV. Response to Leptin Administration in Leptin Deficiency

We have reported the dramatic and beneficial effects of daily sc injections of recombinant human leptin leading to a reduction in body weight and fat mass in three congenitally leptin-deficient children (11, 16). We have recently commenced therapy in two other children and seen comparably beneficial results (our personal observations) (Fig. 1). All



FIG. 1. Effects of recombinant human leptin treatment in leptin deficiency.

children showed a response to initial leptin doses that were designed to produce plasma leptin levels at only 10% of those predicted by height and weight (*i.e.*, approximately 0.01 mg/kg of lean body mass). Leptin therapy has also been successfully used in the three Turkish leptin-deficient adults (20).

The major effect of leptin was on appetite with normalization of hyperphagia. Leptin therapy reduced energy intake during an 18-MJ *ad libitum* test meal by up to 84% (5 MJ ingested before treatment *vs.* 0.8 MJ after treatment in the child with the greatest response) (11). Leptin treatment was associated with reduced hunger scores with no change in satiety in adults with leptin deficiency (20). We were unable to demonstrate a major effect of leptin on basal metabolic rate or free-living energy expenditure, but, because weight loss by other means is associated with a decrease in basal metabolic rate, the fact that energy expenditure did not fall in our leptin-deficient subjects is notable.

The administration of leptin permitted progression of appropriately timed pubertal development in the single child of appropriate age and did not cause the early onset of puberty in the younger children (11). In adults with leptin deficiency, leptin induced the development of secondary sexual characteristics and pulsatile gonadotropin secretion.

In the three previously reported children, there were small, but sustained, increases in free T_4 , free T_3 , and TSH that occurred within 1 month of leptin therapy. These observations are fully consistent with an effect of leptin at the hypothalamic level. A fourth patient had substantial elevation of TSH before treatment, such that T_4 therapy was commenced (12). However, replacement therapy was stopped when thyroid function tests normalized after leptin treatment.

V. Partial Leptin Deficiency in Heterozygote Carriers

The major question with respect to the potential therapeutic use of leptin in more common forms of obesity relates to the shape of the leptin dose response curve. We have clearly shown that at the lower end of plasma leptin levels, raising leptin levels from undetectable to detectable has profound effects on appetite and weight. Heymsfield *et al.* (21) administered supraphysiological doses (0.1–0.3 mg/kg body weight) of leptin to obese subjects for 28 wk. On average, some subjects lost weight, but the extent of weight loss and the variability between subjects has led many to conclude

that the leptin resistance of common obesity cannot be usefully overcome by leptin supplementation, at least when administered peripherally. However, it is of interest that there was a significant effect on weight in some subjects with low serum leptin levels, suggesting that leptin can continue to have a dose/response effect on energy homeostasis across a wide serum concentration range. To test this hypothesis, we studied the heterozygous relatives of our leptin-deficient subjects (22). Serum leptin levels in the heterozygous subjects were found to be significantly lower than expected for percent body fat and they had a higher prevalence of obesity than seen in a control population of similar age, sex, and ethnicity (22). Additionally, the percentage of body fat was higher than predicted from their height and weight in the heterozygous subjects compared with control subjects of the same ethnicity (22). These findings closely parallel those in heterozygous *ob/-* and *db/-* mice (23). These data provide further support for the possibility that leptin can produce a graded response in terms of body composition across a broad range of plasma concentrations.

VI. Complete POMC Deficiency

In 1998, Krude *et al.* provided the first description of humans congenitally lacking proopiomelanocortin (POMC) gene products (24). One proband was a compound heterozygote for two nonsense mutations, and a second patient was homozygous for a mutation in the 5'-untranslated region that introduced an additional out-of-frame start site, thus interfering with POMC translational initiation (Fig. 2). Subsequently, Krude *et al.* (24) have reported three additional unrelated European children with congenital POMC deficiency who were either homozygous or compound heterozygous for POMC mutations (Fig. 2). We have recently identified a sixth patient with complete POMC deficiency, being homozygous for a complete loss of function mutation that results in the loss of all POMC-derived peptides (25).

These patients all presented in early life with features of hypocortisolemia secondary to ACTH deficiency, leading to hypoglycemia, prolonged jaundice, susceptibility to the effects of infection and, in one case, neonatal death. The children responded well to physiological replacement with glucocorticoids but all subsequently developed marked obesity in association with hyperphagia. In this disorder, in both humans and murine models, obesity occurs despite profound glucocorticoid deficiency, a condition normally associated with severe weight loss. In our patient, weight gain

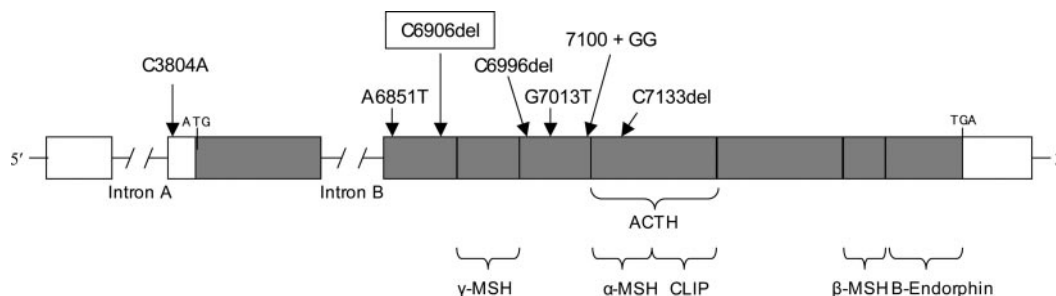


FIG. 2. Mutations in POMC resulting in a complete loss of function.

was documented before the commencement of hydrocortisone replacement therapy. Notably, in *pomc* null mice, restoration of relatively normal glucocorticoid levels results in a marked worsening of the obesity and insulin resistance (26), suggesting that the glucocorticoid deficiency modulates the severity of the metabolic phenotype.

Notably, all children thus far reported have pale skin and red hair, features consistent with the known role of POMC-derived peptides in the determination of the pheomelanin to eumelanin ratio in melanocytes. Our Turkish proband is the first reported patient with POMC deficiency who does not have red hair (25). It is likely this can be explained by his differing genetic background because the other reported patients were all white Caucasian subjects of European ancestry. The retention of dark hair in this child and his similarly affected deceased sibling indicates that the synthesis of eumelanin in humans is not absolutely dependent on the presence of melanocortin peptides. It can be assumed that, in ethnic groups that are predominantly characterized by dark hair, other genetic variants act to maintain eumelanin synthesis in the absence of POMC-derived ligand, whereas in Northern European races, such eumelanin synthesis is more critically dependent on the presence of such ligands (27).

Thus, the cardinal features of congenital POMC deficiency are isolated ACTH deficiency, hyperphagia, and severe early-onset obesity. Although red hair may be an important diagnostic clue in patients of Caucasian origin, its absence in patients originating from other ethnic groups should not result in this diagnostic consideration being excluded.

VII. POMC Haploinsufficiency

Krude *et al.* (28) have previously attempted to assess the impact of loss of one *POMC* allele in the parents and heterozygous relatives of their probands. They estimated the maximum lifetime BMI SDS in adult *POMC* heterozygotes and suggested that most had a maximum lifetime BMI SDS of 1, which is at the upper end of the normal range (28). We had the opportunity to study a large Turkish consanguineous pedigree with 12 heterozygote carriers and seven wild-type subjects (25). The significantly higher prevalence of obesity/overweight in the carriers provides compelling support for the idea that loss of one copy of *POMC* is sufficient to markedly predispose to obesity.

This is particularly relevant because we and others have described a variety of heterozygous point mutations in *POMC*, including mutations in α - and β -MSH, which sig-

nificantly increase obesity risk but are not invariably associated with obesity.

VIII. POMC Mutations Affecting Specific Melanocortin Peptides

To determine whether missense/nonsense mutations within the melanocortin peptides might predispose to obesity, we screened the coding regions of the *POMC* gene for mutations in over 600 UK Caucasian subjects with severe early-onset obesity. We identified a number of sequence variants in *POMC* in severely obese children. Three of these missense mutations directly affect regions of the *POMC* gene that encode melanocortin peptides (Fig. 3). R236G was identified in three patients but also in two controls. We have previously shown that this mutation disrupts a dibasic cleavage site between β -MSH and β -endorphin, resulting in a β -MSH/ β -endorphin fusion protein that binds to melanocortin 4 receptor (MC4R) but has reduced ability to activate the receptor (29). Its presence in both obese probands and controls reflects previous studies that show that this is not a highly penetrant cause of inherited obesity but may increase the risk of obesity in carriers.

We identified five unrelated probands who were heterozygous for a rare missense variant in the region encoding β -MSH, Tyr221Cys (30). This frequency was significantly increased ($P < 0.001$) compared with the general UK Caucasian population and the variant cosegregated with obesity/overweight in affected family members. The overrepresentation of this mutation in obese subjects is supported by independent studies in a German population (31). Compared with wild-type β -MSH, the variant peptide was impaired in its ability to bind to and activate signaling from the MC4R (30). Obese children carrying the Tyr221Cys variant were hyperphagic and showed increased linear growth, both of which are features of MC4R deficiency. These studies support a role for β -MSH in the control of human energy homeostasis.

Interestingly, we found a missense mutation in α -MSH in a single proband, which had a major deleterious effect on its function (30). However, this variant was found in one lean family member and one lean unrelated control. Although it is likely that this variant is contributing to the obesity of the proband, it is notable that our studies provide more compelling evidence for a specific role for β -MSH than α -MSH in the control of human energy balance.

It is possible that an important role for β -MSH in the

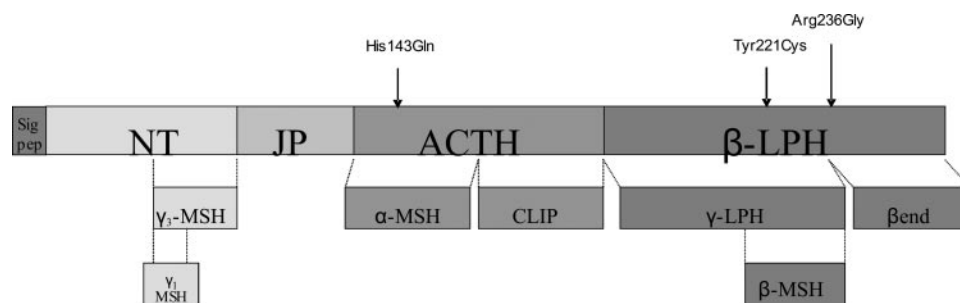


FIG. 3. Mutations in *POMC*-derived melanocortin peptides.

control of energy balance has been overlooked because attention has been principally focused on α -MSH as the probable endogenous ligand in rodents (32). This is largely because rodents lack the proximal dibasic site that is necessary for the proteolytic cleavage event that produces β -MSH in humans. Analogs of β -MSH have been shown to have beneficial effects *in vitro* and *in vivo* in mice (33) and may offer a realistic therapeutic option in these patients.

IX. Genetic Variation at the POMC Locus and Common Obesity

It is notable that a number of genetic linkage studies have identified chromosome 2p22 (a region encompassing the *POMC* gene) as the site of a gene or genes influencing common obesity and obesity-related traits. The strongest evidence for a quantitative trait locus influencing obesity-related phenotypes comes from the San Antonio Family Heart Study undertaken in Mexican-American extended families, with a LOD score of 7.5 for serum leptin levels on chromosome 2p22 (34). Strong evidence for linkage of plasma leptin levels, one of the most robust markers of fat mass, to this region of chromosome 2 was also seen in a genomewide scan performed in French obese sibling pairs (35). Association studies of the *POMC* gene and indices of adiposity have been inconsistent (36, 37), but most have been underpowered. The extent to which these effects are the consequence of variation in or around the *POMC* locus itself has yet to be determined, but the knowledge that the control of human energy balance is sensitive to *POMC* gene dose strengthens the candidacy of *POMC* as a site where variants affecting expression could influence body weight.

It is plausible that genetic variation around the *POMC* locus might confer a risk of obesity through a gene-environment interaction. We recently reported that 129 mice heterozygous for a null mutation in the *pomc* gene became significantly hyperphagic and obese on a high-fat diet but not on normal chow (38). Interestingly, in a recent genomewide scan analysis in Mexican-American families, suggestive evidence of linkage with saturated fat intake was found on chromosome 2p22 (39).

X. Mutations in Prohormone Convertase 1

Many biologically inactive prohormones and neuropeptides are cleaved by serine endoproteases to release biologically active peptides. The prohormone convertases (PC1 and PC2) are expressed in neuroendocrine tissues and act upon a range of substrates including proinsulin, proglucagon, and POMC (40). PC1 is itself synthesized as an inactive precursor, then undergoes two autocatalytic events, first within the endoplasmic reticulum and then within the secretory vesicles of the regulated secretory pathway to generate a fully active 66-kDa isoform that is stored in mature secretory granules.

We have previously reported an adult female with severe early-onset obesity, hypogonadotropic hypogonadism, postprandial hypoglycemia, hypocortisolemia, and evidence of impaired processing of POMC and proinsulin (41). She was found to be a compound heterozygote for *PC1* mutations:

Gly⁵⁹³Arg, which causes failure of maturation of the inactive propeptide form of PC1 (pro-PC1) and its retention in the endoplasmic reticulum; and A \rightarrow C⁺⁴ in the donor splice site of intron 5, resulting in exon skipping, a frameshift, and a premature stop codon in the catalytic domain (42). We have described the second case of congenital PC1 deficiency in a patient who was a compound heterozygote for two loss of function mutations: Glu²⁵⁰ stop, which is predicted to truncate the PC1 protein within the catalytic domain; and Ala²¹³ del, which deletes a highly conserved alanine residue near the catalytically essential His²⁰⁸ residue (43). Intriguingly, this patient suffered from severe small intestinal absorptive dysfunction as well as the characteristic severe early-onset obesity, impaired prohormone processing, and hypocortisolemia. We hypothesized that the small intestinal dysfunction seen in this patient and, to a lesser extent, in the first patient we described may be the result of a failure of maturation of propeptides within the enteroendocrine cells and nerves that express PC1 throughout the gut. The finding of elevated levels of progastrin and proglucagon provided *in vivo* evidence that prohormone processing in enteroendocrine cells was abnormal (43).

PC1 deficiency has been described in two independent mouse models with notable differences in phenotype. In one mouse model, about 40% of *PC1* null embryos die before birth, and another 40% within 6 d. The remaining pups appear normal at birth but are only 60% of the size of heterozygous or wild-type littermates with reduced growth associated with decreased levels of GH mRNA and decreased circulating GH (44). They suffer from chronic mild diarrhea associated with bulky moist stools. Blood glucose levels are normal despite a severe impairment in proinsulin processing, which results in accumulation of immature secretory granules in the pancreatic β -cells. However, these *pc1* null mice were not reported to be obese, leading to the suggestion that PC1 may serve different functions in rodents compared with humans. Recently, a second mouse model of *pc1* deficiency has been generated spontaneously by random mutagenesis, resulting in a homozygous missense mutation (N222D) in the catalytic domain (45). These *pc1* knockouts are hyperproinsulinemic and are 30% heavier than wild-type littermates with an increase in food intake.

An important caveat regarding prohormone processing disorders is that it is very difficult to state with certainty which altered phenotypes are due to which altered processing events. This uncertainty derives from the fact that these enzymes act on multiple precursors, some of which may not as yet be fully characterized.

XI. Human MC4R Deficiency

Of the five known melanocortin receptors, the MC4R has been most closely linked to controlling energy balance in rodents (46). Mice homozygous for a deleted MC4R become severely obese; heterozygotes have body weights intermediate between wild-type and homozygote null animals (47). In 1998, two groups reported heterozygous mutations in the MC4R in humans that were associated with dominantly inherited obesity (48, 49). Since then, heterozygous mutations

in MC4R have been reported in obese humans from various ethnic groups (50–52).

We have studied more than 2000 severely obese probands and found that approximately 5–6% have pathogenic MC4R mutations that are nonconservative in nature, not found in control subjects from the background population, and cosegregate with obesity in families (53). The prevalence of MC4R mutations has varied from 0.5% of obese adults to 6% in patients with severe childhood obesity (53, 54). Recent studies provide an important indication of the true population prevalence of this disorder: 1–2.5% of people with a BMI greater than 30 kg/m² being found to harbor pathogenic mutations in MC4R in UK and European populations (54), confirming that MC4R deficiency is the most common obesity syndrome described to date and is one of the most common genetic diseases with a higher prevalence than more familial diseases such as cystic fibrosis.

Although we found a 100% penetrance of early-onset obesity in heterozygous probands, others have described obligate carriers who were not obese (51). Given the large number of potential influences on body weight, it is perhaps not surprising that both genetic and environmental modifiers will have important effects in some pedigrees. Indeed, we have now studied six families in whom the probands were homozygotes, and in all of these, the homozygotes were more obese than heterozygotes (53). Interestingly, in these families, some heterozygous carriers were not obese. Taking account of all of these observations, codominance, with modulation of expressivity and penetrance of the phenotype, is the most appropriate descriptor for the mode of inheritance. This finding is supported by the pattern of inheritance of obesity seen in heterozygous and homozygous MC4R knock-out mice (47).

We have now studied more than 150 MC4R-deficient subjects in our Clinical Research Facility. The clinical features of MC4R deficiency include hyperphagia, which invariably starts in the first year of life (53). Alongside the increase in fat mass, MC4R-deficient subjects also have an increase in lean mass and a marked increase in bone mineral density, thus they often appear “big-boned.” They exhibit accelerated linear growth in early childhood, which does not appear to be due to dysfunction of the GH axis and may be a consequence of the disproportionate early hyperinsulinemia seen in these patients (53). The accelerated linear growth and the disproportionate early hyperinsulinemia are consistent with observations in the MC4R knockout mouse (55).

Although affected subjects are objectively hyperphagic, *ad libitum* energy intake at a test meal is not as severe as that seen with leptin deficiency (53). Of particular note is the finding that the severity of receptor dysfunction seen in *in vitro* assays can predict the amount of food ingested at a test meal by the subject harboring that particular mutation (Fig. 4). One notable feature of this syndrome is that the severity of many of the phenotypic features appears to partially ameliorate with time. Thus, obese adult mutation carriers report less intense feelings of hunger and are less hyperinsulinemic than children with the same mutation (our personal observations).

We have studied in detail the signaling properties of many of these mutant receptors, and this information should help to advance the understanding of structure/function rela-

Ad libitum energy intake (kJ/kg lean mass)

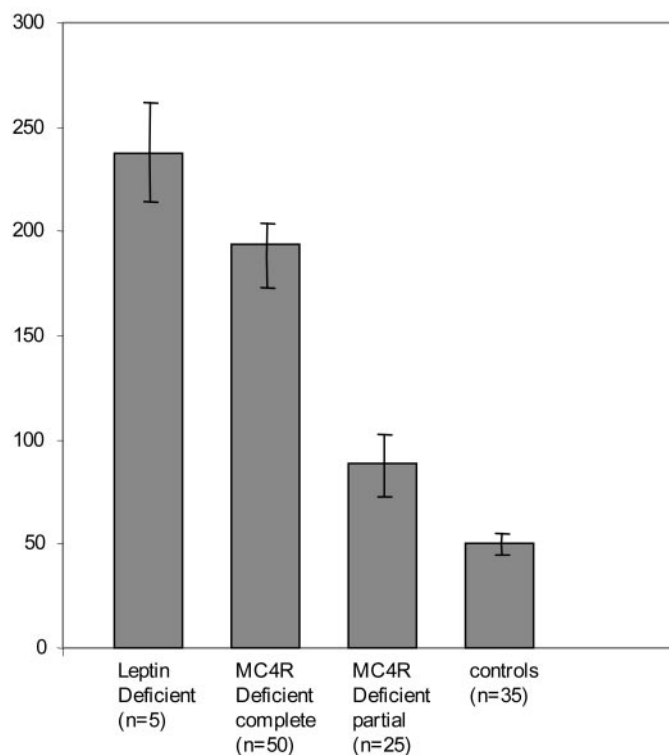


FIG. 4. Genotype-phenotype correlations in human MC4R deficiency. *Ad libitum* food intake at an 18MJ test meal for patients with leptin deficiency and complete and partial loss of function MC4R mutations.

tionships within the receptor (56). Importantly, we have been unable to demonstrate evidence for dominant negativity associated with these mutants, which suggests that MC4R mutations are more likely to result in a phenotype through haploinsufficiency (56). About 70% of missense mutations in MC4R are retained intracellularly (57).

Although, at present, there is no specific therapy for MC4R deficiency, it is highly likely that these subjects would respond well to pharmacotherapy that overcame the reduction in the hypothalamic melanocortineric tone that exists in these patients. Because most patients are heterozygotes with one functional allele intact, it is possible that small molecule MC4R agonists might, in future, be excellent treatments for this disorder (58).

XII. Mutations in the Neurotrophin Receptor TrkB

Recently, the concept that hypothalamic neuronal networks involved in energy homeostasis are “hardwired” has been challenged. In mice, hypothalamic neurones projecting from the arcuate nucleus to the paraventricular nucleus develop after birth, and their development is regulated by leptin (59). In addition, synaptic plasticity in the mature rodent brain has been identified as a component of the neuronal regulation of energy homeostasis because leptin has been shown to acutely modulate excitatory and inhibitory synaptic inputs at the level of first-order arcuate neurones (60). However, it is difficult to establish whether synaptic plasticity plays a role in the physiological regulation of en-

ergy homeostasis in humans and whether under pathological conditions, hypothalamic neuronal networks and plasticity may be impaired and contribute to human obesity.

Brain-derived neurotrophic factor (BDNF) regulates the development, survival, and differentiation of neurons through its high-affinity receptor, tropomyosin-related kinase B (TrkB). Unlike other neurotrophins, BDNF is secreted in an activity-dependent manner that allows for highly controlled release. Recently, BDNF has been implicated in the regulation of body weight because its expression is reduced by fasting (61) and BDNF administration causes weight loss in wild-type mice through a reduction in food intake. BDNF has also been implicated in memory and a range of behaviors using a number of conditional knockout models (62).

We previously reported a child with severe obesity, impaired short-term memory, and developmental delay who had a *de novo* missense mutation impairing the function of TrkB, the tyrosine kinase receptor that mediates the effects of both BDNF and the neurotrophin, NT4/5 (63). We have also identified a patient with severe hyperphagia and obesity and a complex neurobehavioral phenotype including impaired cognitive function and memory as well as distinctive hyperactive behavior. Interestingly, this patient has a *de novo* paracentric inversion, 46,XX,inv(11)(p13p15.3), which encompasses the BDNF locus and disrupts BDNF expression (64). Although to date only two such patients have been identified, understanding the mechanisms whereby BDNF regulates hypothalamic neuronal circuits may have potential therapeutic benefits for the treatment of more common forms of human obesity.

XIII. Conclusions

In practical terms, the discovery of these genetic disorders has helped destigmatize human obesity and allow it to be seen as a medical condition rather than simply a moral failing. The clinical evaluation of the severely obese child is becoming increasingly sophisticated and will require the development of expertise in the recognition of these emerging syndromes together with the incorporation of novel biochemical and molecular genetic diagnostics. These approaches will need to be combined with the more traditional nutritional and behavioral approaches to optimize treatment for individual children. Although there is no accepted definition for severe or morbid obesity in childhood, a BMI SDS greater than 2.5 (weight off the chart) is often used in specialist centers, and the crossing of major growth percentile lines upward is an early indication of risk of severe obesity. The assessment of severely obese children and indeed adults should be directed at screening for potentially treatable endocrine and neurological conditions and identifying genetic conditions so that appropriate genetic counseling and in some cases treatment can be instituted (65). The presence of hyperphagia and severe obesity in a young child (<5 yr old) and a positive family history of early-onset obesity support a genetic diagnosis.

The genetic defects found to date all affect the drive to eat, resulting in hyperphagia in affected subjects. Thus, human food intake should not be considered as an entirely volun-

tarily controllable phenomenon, but rather one driven by powerful biological signals. It is likely that further discovery of causative genetic defects in humans and experimental animals will continue to highlight other molecular elements of the pathways involved in the regulation of body weight. To date, candidate gene studies in the GOOS cohort have led to the identification of seven monogenic disorders, including the most recent disorders involving the neurotrophin receptor TrkB and its ligand, BDNF. However, these disorders only account for 7% of patients in the GOOS cohort (mean BMI SDS, 4.5; mean age of onset, 5 yr). Many of the remaining patients have a history of early-onset obesity inherited in a Mendelian manner or have subphenotypes that overlap with those seen in the known monogenic obesity syndromes, suggesting that there are many other genes and gene products to identify and characterize, and we are undertaking a number of approaches to find novel obesity genes. There are many patients from consanguineous families in whom severe obesity segregates in an autosomal recessive manner and in whom mutations in the known obesity genes have been excluded. The development of high-density single nucleotide polymorphism microarrays and their application in autozygosity mapping provides an efficient way to identify obesity genes in such pedigrees. Comparative genomic hybridization arrays can be used to identify subtle chromosomal rearrangements (less than 5 Mb) that cannot be identified by conventional karyotyping. We are using these arrays in patients with severe obesity, developmental delay, and structural/dysmorphic features. These patients are often uniquely affected in their families, which can be indicative of a *de novo* mutation or rearrangement.

Progress in the discovery of the genetic factors underlying more common forms of obesity, largely of onset in adolescence or adulthood, has not been so swift. It is likely that the effects of any individual genetic variant will be more subtle, and therefore proving its association with an alteration in body weight will be more challenging. In fact, studies of this nature, involving populations of sufficient size to address these questions, have only begun to be undertaken. It is not yet clear whether the genetic architecture of common obesity will conform more to the "common variant–common disease" model in which some relatively common polymorphisms have modest but widespread effects on risk or the "multiple rare variants–common disease" model where multiple different rare alleles underlie genetic susceptibility. It is likely that common genetic variants will selectively influence an individual's response to environmental stimuli and that those of a particular genotype, for example, will be less likely to respond to particular subtypes of dietary intervention than others. Knowledge of gene–environment interaction will increasingly play a role in the improved targeting of behavioral interventions for the prevention of obesity.

Another approach to identifying genes implicated in the regulation of body weight may be to look at the opposite end of the spectrum. Marked thinness appears to be a trait that is as stable and heritable as obesity (66), and evidence from animal models suggests that searching for genes influencing adiposity may be profitably pursued by studying very thin healthy individuals (67). The identification of such genes may provide important insights into the pathophysiological

aspects of obesity and could help explain why some individuals do not become obese despite environmental provocation. It will be interesting to see whether insights into the biology of thinness and obesity resistance may ultimately lead to preventative and management strategies for obesity.

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